# STUDIES OF THE RESPIRATORY METABOLISM OF WARM AND COOL SPRING FISHES ${ }^{1}$ 

F. B. SUMNER AND URLESS N. LANHAM

## I. Introduction

It is generally agreed that the respiratory metabolism (oxygen consumption) of poikilothermal animals tends to vary directly with the temperature to which they are subjected. Less familiar is the existence of a process of thermal adaptation, following transfer from one temperature to another. The immediate effects of such transfer may be succeeded by a "rebound" from the extreme condition first induced back toward the previous condition of the animal. A further illustration of the same principle is the fact that the metabolic rate of fishes at any given temperature may be considerably influenced by their previous temperature experience. Fishes which had been acclimatized to $30^{\circ}$, for example, showed a lower rate of oxygen consumption, when transferred to $20^{\circ}$, than ones which had been kept continuously at $20^{\circ}$, and conversely, transfer from $10^{\circ}$ to $20^{\circ}$ resulted in a higher rate than that shown by fishes accustomed to the latter temperature. Here, too, any such departure from the normal oxygen consumption for a given temperature is followed, within a few days, by a process of adaptation which tends to equalize the metabolic rate, regardless of previous history (Wells, 1935b; Sumner and Wells, 1935 ; Sumner and Doudoroff, 1938).

An obvious question is whether the metabolic rate of fishes and other poikilothermal animals in nature varies as widely with the temperature of their medium as might be inferred from these laboratory results. Or, may not more far-reaching processes of adaptive modification occur in nature, such that warm-water and cold-water derivatives of the same stock come to have nearly the same rates of metabolism?

Fox and Wingfield ${ }^{2}$ compared certain closely related species, or local representatives of the same species, living at different latitudes, and reported varying degrees of thermal adaptation of this sort. In some cases, there was no such adaptation, the two forms under comparison differing widely under their own normal conditions of life, but approximating one
${ }^{1}$ Contributions from the Scripps Institution of Oceanography of the University of California, La Jolla, California, New Series No. 157. Services were rendered in the course of these studies by persons working under W.P.A. Project 65-1-07-2317.
${ }^{2}$ Fox, 1936, 1939a, 1939b; Fox and Wingfield, 1937; Wingfield, 1939.
another closely when their rates of physiological activity were compared at equal temperatures. In other cases, the rates of these functions were nearly identical in their respective habitats, despite considerable differences of temperature. Here extensive adaptation appears to have taken place.

In 1939 Sumner and Sargent made field studies of the respiratory metabolism of fishes in springs of varying temperature in Nevada. Experiments were conducted upon warm and cool spring representatives of species belonging to two genera, Cyprinodon and Crenichthys (Sumner and Sargent, 1940). In these studies, the rapidity of death in thousandthnormal KCN was used as an index of the rate of respiratory metabolism, the two, as is well known, being directly correlated.

In both of these genera, the warm-spring fishes, at their accustomed temperature, succumbed far more rapidly than the cool-spring fishes at their accustomed temperature. As regards the behavior of warm-spring individuals, transferred to the cool spring, the two fishes seemed to give different results, though the data hardly warrant definite conclusions on this point. It should be pointed out, however, that the seeming lack of adaptive modification of the metabolic rate on the part of Crenichthys in warm water has been borne out by the studies to be reported herewith. Its striking resistance to the lethal effects of high temperature is something quite distinct (Sumner and Doudoroff, 1938).

The importance of the physiological and ecological problems here involved, and the widespread human threat to the continued availability of warm springs to naturalists (Brues, 1928) seemed to warrant a second trip to the springs previously visited in our studies of Crenichthys. This work was carried on during portions of September and October, 1941.

While we believe that the publication of our present results calls for no apologies, it should be pointed out that field studies of this sort should not be judged by the same standards as are applied to similar studies in the laboratory. This is because of the impossibility of transporting important articles of laboratory equipment, unfavorable weather conditions, and limited time (in this case, less than 4 weeks) at the scene of our operations.

We must here acknowledge valuable help received at various times from our colleagues, Dr. Denis L. Fox and Dr. Marston C. Sargent, and from our companion in camp, Mrs. Margaret Sumner.

## II. Methods

Altogether, 200 fishes were employed in these studies, while more than 200 oxygen determinations were made. The fishes were readily caught
at both of these springs with hand-nets. Some time (several hours to a day or more) before the respiration tests were made, small lots ( 6 to 9 ) of the fishes, of roughly uniform size, were placed in each of four glass tubes having a length of 40 cm . and an internal diameter of 4 cm . Except during the period of a test, wide-mesh cloth screen was fastened over the flanged ends of the tubes, which were laid, side by side, in a screen cage, placed at the bottom of the stream, and directly in the line of the current. When a test was to be made the screens were removed from one of the tubes, and close-fitting rubber stoppers, one of these carrying a glass stopcock, were forced into its ends. The tube was then returned to its former position in the cage. The capacity of these tubes, with the stoppers in place, ranged from 439 to 454 ml .

At the close of 15 minutes ( 10 in some experiments) the watersamples were drawn off into glass-stoppered bottles of about 150 ml . capacity. A glass tube, continuous with the stopcock, was inserted into the bottle, down to its bottom, and the water allowed to overflow from the bottle's mouth. Two samples were invariably drawn in each test. ${ }^{3}$ Time records, in seconds, were kept of each of these steps with a stopwatch. Within a few minutes of the drawing of each sample, the Winkler reagents were introduced into the bottles. Titration was performed on either the same or the next day. ${ }^{4}$

In recent studies of oxygen consumption in fishes (cf. Keys, 1931 ; Wells, 1935a; Wunder, 1936; Leiner, 1938) it has been the more usual practice to hold the animals in a continuous current of water, and to draw samples from this at intervals, rather than to hold them, tightly stoppered, in an inclosed volume of water. Under the former conditions, the accumulation of carbon dioxide and other possible metabolites is, of course, prevented. Likewise the fishes are subjected to less disturbance in the taking of water samples. It is obvious, however, that such procedure would be difficult, if not impossible, to employ in the field. Moreover, the highest of our $\mathrm{CO}_{2}$ concentrations probably fell well short of two thousandths of an atmosphere. No signs of discomfort were visible at the close of the 15 -minute period, at least in the cooler water. So far as disturbance is concerned, the fishes showed little evidence of excitement except at the beginning and the end of the test. Most of the time they swam quietly or remained at rest. In this connection, it is worth pointing

[^0]out that our figures show no more variability for fishes of a given size than do those of Wells (1935a, pp. 209-210) in experiments with Fundulus, where a rumning-water system was employed. Indeed Wells has recorded fully as great differences as ours when repeating tests of the same lots of fishes.

Before and after the performance of a series of these tests, and frequently alternating with them, water samples were withdrawn from the spring, as near as possible to where the tubes had lain.

Owing to the possible presence of nitrites in our samples (cf. Allee and Oesting, 1934) the modified Winkler method (A.P.H.A., 1936) ${ }^{5}$ was used in all cases, with a few exceptions which were performed for the sake of comparison. ${ }^{6}$

Under field conditions, it is hardly to be expected that such high precision will be attained as is possible in the laboratory. Thirty cases in which duplicate water samples were drawn from a single tube revealed a mean difference of 2.2 per cent between them. Lack of homogeneity ("streakiness"), in the water, to be discussed presently, may have had its effect, even within our half-liter tubes.

At the close of each test of oxygen consumption, the fishes involved were dried upon paper towels and weighed. The aggregate weight of each lot, together with the estimated original oxygen titre of the water, the oxygen titre at the close of the test, the volume of water in the tube (allowing for volume occupied by the fishes), and the exact duration of the test, are the data upon which the metabolic rates (ml./gm./hr.) are based.

Unfortunately, the equipment at our disposal did not permit of our collecting a sample of the water admitted to the tube at the moment of commencing each test of oxygen consumption. For this reason, the previous oxygen titre of the water which was actually "breathed" by the fishes in any given experiment cannot be stated with precision. As will appear later, water samples from these springs, taken at different times or in slightly different positions, might differ considerably in their oxygen titre. While this circumstance introduces a considerable margin of possible error into the result of any single titration, we believe that the validity of our chief comparisons is little affected. This (1) because the
${ }^{5}$ Our procedure differed from the procedure therein stated in that in the succeeding steps of the test (Winkler proper) HCl was used instead of $\mathrm{H}_{2} \mathrm{SO}_{4}$, while manganous chloride was used in place of the sulphate. This is in accordance with the procedure long employed at the Scripps Institution in analysis of sea-water.
${ }_{6}$ It is doubtful whether the modified procedure was necessary, in the case of the water-samples taken directly from the springs. Four divided samples, of which half of each was subjected to the modified and half to the unmodified Winkler procedure, gave mean values of 2.54 and 2.55 respectively.
differences involved in comparing two series of differing history are great in proportion to errors due to uncertain original oxygen content of the water; and (2) because results based upon two different procedures which we have employed in selecting a figure to represent this oxygen content show substantially the same relations.

The most obvious of these procedures (1) was to select for each computation the oxygen value of that water sample which was taken most nearly at the same time as the performance of the metabolism test under consideration. However, there were instances in which no water sample was taken within an hour or more of this test.

The alternative procedure (2) was to employ, as our basis of calculation, the mean titre of all the water samples taken in the position in which the test in question was made. ${ }^{7}$ It is obvious that many single values for oxygen consumption obtained in this way would be no more than rough approximations. It is equally plain, however, that the average of a series of values thus obtained could not depart far from an average such as we should obtain if the actual oxygen titres of the corresponding water samples were available.

It is gratifying that graphs based upon these two procedures agree in most essential features, though differing considerably in detail (Figs. $2-4)$. In the text, the mean of the two resulting values will be given.

A further difficulty in postulating any initial oxygen content for the water is the fact that a quite appreciable amount of oxygen was taken out of their medium by the fishes before the commencement of the test, despite the continuous flow of water through the tubes. This current was undoubtedly retarded somewhere by the screens, which it was necessary to fasten over the ends. Whatever the exact extent of this preliminary depletion of the oxygen, we may probably fairly assume that it did not differ very widely from one experiment to another.

## III. The Springs

"Preston Spring," the largest of several springs in the vicinity of Preston, Nevada, is really a group of small springs, discharging near together and giving rise to a creek of considerable size. The water in and near the orifices appears to maintain a constant temperature very close to $21^{\circ} \mathrm{C}$.

The high variability of the oxygen values for all of the water samples taken at Preston Spring is indicated by Fig. 1. Reference has already been made to the "streakiness" of this water in respect to oxygen content.

[^1]While the concentration at the sources appears to be fairly constant (cf. Sumner and Sargent, 1940), it is considerably lower than that in the rest of the stream, even in their immediate neighborhood. Surface aeration and photosynthesis are, of course, responsible for the differences. The mean of two samples, drawn from what appeared to be the largest orifice was 1.96 ml . per litre, while samples taken a few yards away averaged about 2.60 ml . The resulting variegation in the oxygen concentration of these waters was shown by determinations of samples taken in various positions.

It would be quite erroneous, however, to infer that the water with which we filled our tubes for the tests of respiratory metabolism was

1.92 .02 .12 .22 .32 .42 .52 .62 .72 .82 .9

Fig. 1. Oxygen values ( $\mathrm{ml} . / 1$.) for water of Preston Spring. Shaded squares denote samples taken in the first position of our cage directly over one of the minor orifices of the spring; the others in a second position some distance from this.
subject to such an extreme range of variation as is here indicated. Care was taken, moreover, to collect our samples from points close to those where the tests were made.

Some thirty miles to the south, in the same valley. ${ }^{8}$ is the warm spring here discussed, known locally as "Mormon Spring." This is a spring of much smaller volume than that at Preston. Its temperature is almost exactly $37^{\circ}$ at its source, but the water cools off by a degree or two within a hundred feet downstream. Most of the fishes were caught in water ranging from $35^{\circ}$ to $36.5^{\circ} \mathrm{C}$., and temperatures such as these prevailed where the experiments were performed. At the lowest point in the stream where fishes were seen, the thermometer recorded $34.8^{\circ}$. Within

[^2]a few hundred feet from its source, the stream spreads out into a marsh, where the water probably follows the temperature of the air. No fishes were found there. It seems probable, therefore, that the Mormon Spring representatives of Crenichthys baileyi se!dom experience temperatures lower than $34^{\circ}$. On the other hand, individuals were rather frequently seen close to the main source, where the temperature was nearly or quite $37^{\circ}$.

As regards oxygen content, water samples taken well below the surface, in the main orifice of the spring, gave a mean value of $0.51 \mathrm{ml} . / 1$., while at a nearby point, where fishes were often seen, this value was 0.65 . Samples taken downstream in the creek, where most of the fishes were caught, ranged from 1.54 to $1.88 .{ }^{9}$

## IV. The Fishes

Crenichthys baileyi (Gilbert) ${ }^{10}$ is the commonest species of fish in Preston Spring, at least near the sources. Besides Crenichthys, there are, however, at least three other fishes present. Two of these (Apocope osculus and Lepidomeda sp.) are Cyprinidae, the other (Pantosteus sp.) being a Catostomid. At Mormon Spring, Crenichthys is the only fish present. Likewise, it is much less abundant than in Preston Spring. Dr. Hubbs informs us that he regards the two populations of Crenichthys here considered as subspecifically distinct. He states (cf. Hubbs and Kuhne, 1937, referring to similar differences in Apocope) that the warm spring form has a slightly lower average number of fin-rays than the cool spring form, and counts which we have made of the dorsal and anal rays of about fifty fishes of each race confirm this statement.

Identification of the sexes is difficult or impossible in the smaller specimens mostly used in these studies. Since such individuals were probably sexually immature, it is not likely that sexual differences occurred in their metabolic rate.

## V. Oxygen Consumption

Experiments were performed in which each stock was tested in its own waters, and in which each was tested after transfer to the habitat of the other.
Preston at Preston

When all of these fishes are thrown together as a single population (Fig. 2 and Table I), the values for respiratory metabolism (ml./gm.-
${ }^{9}$ Readings for these were as follows: 1.54, 1.56, 1.62, 1.76, 1.78, 1.86, 1.86, 1.88.
${ }^{10}$ Cf. Hubbs, 1932; Hubbs and Miller, 1941. We are indebted to Dr. Hubbs for the identification of the other species.
/hr.) are seen to vary very widely, ranging from 0.123 to 0.390 , using in each case the mean of the values obtained by our two procedures (see p. 317). This variation is in part due to the heterogeneous nature of the material. The series includes fishes differing widely in size, this ranging from 0.51 gm . (mean of one lot of 9 individuals) to 9.5 gms . (weight of one large individual tested alone). While the (inverse) correlation between size and metabolic rate is not very close, Figs. 2 and 3 show that


Fig. 2. Rate of oxygen consumption (ml./gm./hr.) of Preston Spring fishes in Preston Spring. Upper histogram is based upon computations (1) in which the $\mathrm{O}_{2}$ value of the water sample taken at most nearly the same time as the test was used in each case; lower histogram upon computations (2) in which the mean of the entire series of $\mathrm{O}_{2}$ values (with certain exceptions) was used (see p. 317). Shaded squares denote rate for fishes larger than 1 gram in mean weight, others for fishes less than 1 gram.
such correlation exists. It will be found, too, that the mean of ten values for fishes averaging over 1 gm . in weight is 0.215 , that of the thirteen values for fishes below this mean weight being 0.307 (see Fig. 2).

In two cases, special experiments were made with Preston-at-Preston lots, in order that more strict comparisons with the Mormon-at-Mormon series might be possible. Thus, in the case of four lots (designated by asterisks) the period of the test was abbreviated to ten minutes (see be-
low) instead of fifteen minutes as was the rule in the Preston Spring fishes. Again, four other lots (designated by daggers) were tested only a few hours ( $11 \%$ to 4 ) after being confined in the tubes, instead of being previously kept through one night, as was commonly done. While the results of both of these tests indicated considerable differences from the general average ( 13 to 18 per cent), part of this difference appears to

Table I
Oxygen Consumption of Fishes (First Sample Drawn)—Preston Spring
(Arranged in order of mean weight)

| Lot of | Weightof sample of sample | Numberof fishes | Mean weight | ml./gm./hr. |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Procedure 1 | Procedure 2 | Means |
| A | 4.6 | 9 | 0.51 | 0.370 | 0.424 |  |
| B | 5.1 | 9 | 0.57 | $0.341 \bigcirc 0.337$ | 0.3070 .320 | 0.328 |
| C | 5.1 | 9 | 0.57 | $0.278{ }^{0.337}$ | $0.200{ }^{0.320}$ | 0.328 |
| D $\dagger$ | 3.5 | 6 | 0.58 | 0.358) | $0.348)$ |  |
| E $\dagger$ | 3.8 | 6 | 0.63 | 0.357 | 0.271 | 0.314 |
| F | 6.6 | 9 | 0.73 | 0.253 | 0.293 | 0.273 |
| G | 5.1 | 6 | 0.85 | $0.283{ }^{\text {a }} 0.275$ | $0.212\}_{0.252}$ | 0.264 |
| $\mathrm{G}^{*}$ | 5.1 | 6 | 0.85 | 0.268 | $0.293{ }^{0.252}$ | 0.264 |
| $\mathrm{H} \dagger$ | 5.4 | 6 | 0.90 | 0.309 | 0.303 |  |
| I | 5.4 | 6 | 0.90 | 0.306 | 0.236 |  |
| I'* | 5.4 | 6 | 0.90 | $0.333>0.322$ | 0.343 -0.306 | 0.314 |
| J | 5.5 | 6 | 0.92 | 0.326 | 0.317 |  |
| $\mathrm{J}^{\prime *}$ | 5.5 | 6 | 0.92 | 0.334 | 0.329 |  |
| K | 7.0 | 6 | 1.16 | 0.15330 .248 | 0.222 亿0.230 | 0.239 |
| L $\dagger$ | 7.0 | 6 | 1.17 | $0.343{ }^{0.248}$ | $0.239\}^{0.230}$ |  |
| M | 7.8 | 6 | 1.30 | 0.277 | 0.270 |  |
| $\mathrm{I}^{\prime *}$ | 7.8 | 6 | 1.30 | $0.311\} 0.244$ | $0.308 \bigcirc 0.261$ | 0.252 |
| N | 8.0 | 6 | 1.33 | 0.144 | 0.204 |  |
| O | 8.7 | 6 | 1.45 | 0.236 | 0.222 | 0.229 |
| P | 10.0 | 6 | 1.67 | 0.209 | 0.227 | 0.218 |
| Q | 11.2 | 6 | 1.87 | 0.179 | 0.205 | 0.192 |
| R | 5.4 | 1 | 5.40 | 0.150 | 0.111 | 0.130 |
| S | 9.5 | 1 | 9.50 | 0.136 | 0.153 | 0.144 |
|  | Means |  | 1.56 | 0.272 | 0.262 | 0.267 |

have been due to the size of the fishes employed. In any case the chief relations here considered are little affected.

## (2) Mormon at Mormon

It was difficult for practical reasons (distance and time) to leave the Mormon Spring fishes over night in tubes before making the tests of oxygen consumption in their own habitat, as was customary in the

Preston-at-Preston series. Likewise it was found that the usual fifteen minutes after stoppering was too long a period of confinement, considering the high temperature ( $36^{\circ} \pm$ ) of the water and its low oxygen content.

Two preliminary lots, however, were subjected to this longer test. The values for oxygen consumption for these lots (mean weights 0.76 and 0.67 gm .) were 0.386 and $0.382, \mathrm{ml} . / \mathrm{gm} . / \mathrm{hr}$., respectively. Some of these fishes showed marked signs of asphyxiation during the last few minutes. That respiratory values for partially asphyxiated fishes cannot be regarded as normal is obvious.


Fig. 3. Comparison of "Preston-at-Preston" series with "Mormon-at-Mormon" and with "Mormon-at-Preston" (1 day). Abscissas $=$ mean weight of fishes in each lot; ordinates $=$ oxygen consumption ( $\mathrm{ml} . / \mathrm{gm} . / \mathrm{hr}$.). Solid lines conmect values obtained by second of above procedures (see legend for Fig. 2) ; broken lines connect values obtained by the first procedure.

The remaining four tests, limited to ten minutes, are very instructive. In Table II (upper section) it is seen (1) that values for oxygen consumption are far higher in this series than in the "Preston at Preston" series; (2) that these values are inversely correlated with the mean weights of the fishes involved. This correlation is far more evident than in the Preston series, a significant fact which will be discussed shortly. Comparisons between the Mormon-at-Mormon and Preston-at-Preston series may be made by inspection of Fig. 3. That the relations shown here are little affected when we substitute certain more strictly comparable Preston-at-Preston lots, has already been pointed out.
(3) Preston at Mormon

It had already been reported by Sumner and Sargent (1940) that Preston Spring fishes speedily die when transferred to Mormon Spring. In the more recent experiments, it was found that the fishes were overcome so rapidly by the heat that no instructive determinations of oxygen consumption were possible.
(4) Mormon at Preston

In these experiments, the fishes were transferred from Mormon Spring to Preston Spring in partially insulated bottles. The temperature fell seven or eight degrees in the course of transfer, though this circum-

Table II

|  | Lot of fishes | Weightof sample | Number | Mean weight | ml./gm./hr. |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | Procedure 1 | Procedure 2 | Means |
| Mormon at Mormon | T | 3.5 | 9 | 0.39 | 0.739 | 0.612 | 0.675 |
|  | U | 4.4 | 6 | 0.73 | 0.597 | 0.555 | 0.571 |
|  | V | 5.1 | 6 | 0.85 | 0.528 | 0.544 | 0.536 |
|  | W | 8.3 | 6 | 1.38 | 0.382 | 0.423 | 0.402 |
|  | Means | Same values |  | 0.84 | 0.561 | 0.533 | 0.546 |
| Mormon at Preston ( $2 \frac{1}{2}$ to 4 hours) | T' |  |  | 0.422 |  |  |
|  | $\mathrm{U}^{\prime}$ |  |  | 0.374 |  |  |
|  | $\mathrm{V}^{\prime}$ |  |  | 0.328 | 0.342 | 0.335 |
|  | W' |  |  | 0.255 | 0.263 | 0.259 |
|  | Means | Same values |  |  |  | 0.345 |  |  |
| (1 day) | $\mathrm{T}^{\prime \prime}$ |  |  |  | 0.268 | 0.308 | 0.288 |
|  | $\mathrm{U}^{\prime \prime}$ |  |  |  | 0.256 | 0.288 | 0.272 |
|  | $\mathrm{V}^{\prime \prime}$ |  |  |  | 0.266 | 0.308 | 0.287 |
|  | $\mathrm{W}^{\prime \prime}$ |  |  |  | 0.278 | 0.304 | 0.291 |
|  | Means |  |  |  |  | 0.267 | 0.302 | 0.284 |

stance would seem to have little bearing on the results. Three different series of these fishes ( 8 lots, comprising 57 fishes) were thus transferred. The most instructive of these series consisted of the same four lots as were employed in the principal Mormon-at-Mormon tests. The further history of these fishes is included in Table II, and is portrayed graphically, in part, in Fig. 4. After the test in Mormon Spring, these fishes were left over night in the latter, in the same tubes (screened at the ends). They were then taken in bottles to Preston Spring and tested,
first, after the lapse of $21 / 2$ to 4 hours following transfer; next after a day. As regards their performance after this briefer period, we have complete figures only for the second of our procedures in computing oxygen consumption. The mean of these four values is 0.345 , which is plainly intermediate between 0.561 (before the transfer) and 0.267 (one day following the transfer).

The significant features to be observed in the behavior of these four lots of fishes at the close of a day following transfer are (1) the great fall in metabolic rate in the cooler water ; (2) the inverse correlation be-


Fig. 4. Rate of oxygen consumption of same group of Mormon Spring fishes: (1) at Mormon Spring; (2) after 1 day at Preston Spring. Ordinates $=\mathrm{ml} . /-$ $\mathrm{gm} . / \mathrm{hr}$. Solid and broken lines have same significance as in two preceding figures. Figures opposite brackets denote mean weights of the fishes of these respective lots.
tween the size of the fishes and the rate of this fall, resulting in a marked convergence of the curves for the four size-groups, which come to nearly a common level by the end of 24 hours.

A question of considerable interest is whether the present figures give evidence that the respiratory metabolism of these warm-adapted fishes, when transferred to much cooler water, undergoes any such fall below the rate shown by cool-adapted specimens as had been shown in previous laboratory experiments.

At the close of the day our mean figure for the metabolic rate of six lots of Mormon-at-Preston fishes was 0.284 ; that for the Preston fishes in their natural habitat being 0.267 . The mean weight of the Mormon-at-Preston fishes ( 0.79 gm. ) is, however, much less than that of the Preston-at-Preston fishes ( 1.56 gms .), a fact which might, perhaps, account for this higher rate. Graphs for these two groups (Fig. 3), in which each is plotted according to weight, make it plain that any possible difference of metabolic rate, in either direction, is small in extent.

It cannot, therefore, be affirmed with any probability that these warmspring fishes, when transferred to water at $21^{\circ}$, display a rate of oxygen consumption which is lower, at the close of one day, than fishes native to the latter. This conclusion may be regarded as confirming the results of the earlier test with the cyanide method, already referred to.

Two of the eight Mormon-at-Preston lots comprised in our various experiments were kept in the latter spring for two more days. ${ }^{11}$ The figures for these two lots (mean weight 0.52 and 0.57 grams) were 0.283 and 0.343 . While not very consistent, these figures certainly do not indicate any further fall of metabolic rate.

The convergence of the four pairs of curves in figure 4 is a striking feature of this graph and deserves some comment. At the close of a day in the cool spring, the differences between these four lots, in respect to metabolic rate, have virtually disappeared, or have even been slightly reversed. Thus, the correlation between size and metabolic rate, which is so conspicuous at $36^{\circ}$, has vanished at $21^{\circ}$. It has already been pointed out that this correlation is low in the cool-spring fishes themselves.

A marked divergence of the temperature- $\mathrm{O}_{2}$-consumption curves for "large" and "small" Fundulus is shown by Wells (1935a, Figs. 3 and 4). Wells's observations relate to temperatures ranging from $10^{\circ}$ to $24^{\circ}$, so that the case is not altogether parallel with our findings on Crenichthys, in which we are dealing with water at $21^{\circ}$ and $36^{\circ}$, and with no intermediate values. In the case of our warm-spring fishes, too, the correlation actually disappears at the lower temperature. It is plain, however, that the two cases are in essential agreement in one important respect.

That the metabolic rate of organisms declines with age, or conversely, that a higher metabolic rate is to be expected in younger animals, appears to be an accepted fact (Child, 1915; Heilbrumn, 1937). Since fishes are known to grow more or less continuously through life, it is usually a safe assumption to regard relative size as an index of relative age. Our
${ }^{11}$ Some specimens were kept for four and even six days, but these were weighed in the meantime. Since a number of these fishes died as a probable result of the unavoidable drying, the results cannot be regarded as significant.
series of fishes of increasing weight (Table I) may therefore be safely regarded as representing, on the whole, a series of increasing age.

Concerning the correlation between age and the temperaturecoefficient, less appears to be known. The majority of workers seem to have reported that this coefficient increased with age (Bělehrádek, 1935). Our figures, on the contrary, as well as those of Wells, certainly indicate a higher temperature coefficient for the smaller fishes.

## Summary

Fishes (Crenichthys bailcyi) living in a warm spring at temperatures of $35^{\circ}$ to $37^{\circ}$ have a far higher rate of oxygen consumption ( $\mathrm{ml} . /-$ $\mathrm{gm} . / \mathrm{hr}$.) than fishes of the same species living in a cool spring $\left(21^{\circ}\right)$. The ratio, when fishes of approximately the same weight are compared, is not far from 2 to 1 .

Cool spring fishes, when transferred to the warm spring, die so rapidly that measurements of oxygen consumption are not practicable.

When the converse experiment is performed (transfer of warmspring fishes to the cool spring), they live in health for some days, and may perhaps do so indefinitely. Changes in their rate of oxygen consumption are as follows: (1) A rapid fall occurs within the first few hours. (2) At the close of a day, the metabolic rate (oxygen consumption) is about the same as that of fishes native to the cool spring. If any difference exists, when specimens of the same size are compared, this is probably slight.

This failure of the metabolic rate of warm-spring fishes to fall below that of cool-spring fishes, when transferred to the latter medium, accords with previous experiments at these same springs in which the cyanide method was used, but it does not accord with experiments performed upon some other species in the laboratory.

A marked inverse correlation exists between size (age) and metabolic rate. This correlation is more marked at high temperatures than at lower ones. Thus the curves for the four size-groups of warm-spring fishes here considered converge strongly during the test in cool water, coming together at nearly a common point by the end of the day. This last fact harmonizes with the fact that the correlation between size and metabolic rate, while observable, was found to be low among the cool spring fishes.

The oxygen consumption of smaller fishes is much more affected by temperature changes than is that of the larger specimens. In other words, the temperature coefficient is higher for the smaller (younger) animals, a fact which does not appear to agree with most results which have been reported for other organisms.

## LITERATURE CITED

Allee, W. C., and Oesting, R., 1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. Physiol. Zoöl., 7 : 509-541.
American Public Health Association, 1936. Standard methods for the examination of water and sewage. New York.
Bělehrádek, J., 1935. Temperature and living matter. Berlin: Gebriider Borntracger, 277 pp .
Brues, C. T., 1928. Studies on the fauna of hot springs in the western United States and the biology of thermophilous animals. Proc. Amer. Acad. Arts and Sci., 63: 139-228.
Child, C. M., 1915. Senescence and rejuvenescence. Chicago: Univ. Chicago Press, 481 pp.
Fox, H. M., 1936. The activity and metabolism of poikilothermal animals in different latitudes. I. Proc. Zoöl. Soc. London, pp. 945-955.
--, 1939a (same title). III. Proc. Zoöl. Soc. London, Ser. A, 108: 501-505.
--, 1939 (same title). V. Proc. Zoöl. Soc. London, Scr. A, 109: 141-156.
Fox, H. M., and Wingrield, C. A., 1937 (same title). II. Proc. Zoöl. Soc. London, 107: 275-282.
Heilbrunn, L. V., 1937. An outline of general physiology. Philadelphia: W. B. Saunders, 603 pp .
Hubbs, C. L., 1932. Studies of the fishes of the order Cyprinodontes. XII. A new genus related to Empetrichthys. Occasional Papers of the Muscum of Zology, University of Michigan, No. 252: 1-5.
Hubbs, C. L., and Kuhne, E. R., 1937. A new fish of the genus Apocope from a Wyoming warm spring. Occasional papers of the Muscum of Zoölogy. Unizersity of Michigan, No. 343: 1-21.
Hubbs, C. L., and Miller, R. R., 1941. Studies of the fishes of the order Cyprinodontes. XVII. Genera and species of the Colorado River system. Occasional Papers of the Muscum of Zoölogy, Unizersity of Michigan, No. 433: 1-9.
Keys, A. B., 1931. A study of the selective action of decreased salinity and of asphyxiation on the Pacific killifish, Fundulus parvipinnis. Bull. Scripps Iust. of Oceanog. (Tcch. Ser.), 2: 417-490.
Leiner, M., 1938. Der Physiologie der Fischatmung. Leipzig: Akadcmische Verlagsgesellschaft M.B.H. 134 pp.
Sumner, F. B., and Doudoroff, P., 1938. Some experiments upon temperature acclimatization and respiratory metabolism in fishes. Biol. Bull., 74: 403429.
-, and Sargent, M. C., 1940. Some observations on the physiology of warm spring fishes. Ecology, 21: 45-54.
--, and Wells, N. A., 1935. Some relations between respiratory metabolism in fishes and susceptibility to certain anesthetics and lethal agents. Biol. Bull., 69 : 368-378.
Wells, N. A., 1932. The importance of the time element in the determination of the respiratory metabolism of fishes. Proc. Nat. Acad. Sci., 18: 580-585.
-, 1935a. The influence of temperature upon the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis. Physiol. Zoöl., 8: 196-227.
-, 1935b. Change in rate of respiratory metabolism in a teleost fish induced by acclimatization to high and low temperature. Biol. Bull., 69: 361-367.
Wingrield, C. A., 1939. The activity and metabolism of poikilothermal animals in different latitudes. IV. Proc. Zöl. Soc. London, Scr. A, 109: 103-108.
Wunder, W., 1936. Physiologie der Süsswasserfische Mitteleuropas. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung, 340 pp.


[^0]:    ${ }^{3}$ The values for oxygen consumption employed in our tables and graphs have been based entirely upon the first of these samples. The second figures have been regarded as less representative for a number of reasons.
    ${ }^{4}$ No appreciable effect of delaying the titration over night is probable. Readings of four divided samples, titrated on the same and on the following day, gave mean values of 2.48 and 2.52 respectively. These were as close as the average divided samples titrated on the same day.

[^1]:    ${ }^{7}$ Excluding those taken on cloudy days or near sunset, which regularly gave very low values.

[^2]:    ${ }^{8}$ Called the "White River Valley," though there is no continuous stream here.

